ASEAN
Journal of Scientific and Technological Reports
Online ISSN:2773-8752

Research article

Occurrence of Parasitic and Bacterial Pathogen in Ornamental and Wild Populations of Siamese Fighting Fish (*Betta splendens*) in a Region of Thailand

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Citation:
Manklinniam, P.; Phunpruch, S.; Worananthakij, W. Occurrence of parasitic and bacterial pathogen in ornamental and wild populations of Siamese fighting fish (*Betta splendens*) in a region of Thailand. *ASEAN J. Sci. Tech. Report.* **2025**, *28*(3), e257870. https://doi.org/10.55164/ajstr.v28i3.257870.

Article history:
Received: February 12, 2025
Revised: April 7, 2025
Accepted: April 19, 2025
Available online: April 26, 2025

Publisher's Note:
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Abstract: The diversity of fish parasites reflects the diversity of parasites in the water source, providing insights into the effects of pathogens and essential information about parasite-host relationships. Parasitic infections are valuable indicators of the aquatic ecosystem, influenced by various factors. This study aimed to investigate the prevalence and seasonality of parasite species and pathogenic bacteria in Siamese fighting fish (*Betta splendens*) over 12 months, involving sixty ornamental Siamese fighting fish collected from commercial sources and 81 wild Siamese fighting fish from different natural habitats. Parasite prevalence in ornamental Siamese fighting fish (25.00%) was lower than in the wild (34.57%). The protozoa parasite Trichodina was found in both Siamese fighting fish, with the highest prevalence recorded for *Trichodina* sp. in wild Siamese fighting fish. The winter season exhibited the highest parasitic prevalence, with a tremendous diversity of parasites found at each location, followed by the rainy and summer seasons. This study also reported the first finding of *Henneguya* sp. infection in Siamese fighting fish and on the body surface. The prevalence and seasonality of parasite genera were significant in the wild compared to ornamental Siamese fighting fish. Bacterial isolation was performed on internal organs, and isolates were identified using PCR techniques. *Aeromonas veronii* and *Mycobacterium marinum* were detected in ornamental Siamese fighting fish, while *A. veronii* was found in wild Siamese fighting fish. These findings indicate that infections in Siamese fighting fish display seasonal variation and are impacted by their ecology. This information is fundamental for managing the biodiversity of parasites in fish and preventing parasite infections in aquaculture.

Keywords: Fish parasites; Henneguya; Mycobacteriosis; Seasonality of parasites; Wild Siamese fighting fish

1. Introduction

Siamese fighting fish, known as *Betta splendens* (Regan, 1910), belong to the freshwater fish family Osphronemide, Genus Betta, and are of the reproduction bubble nest type. *B. splendens* is the origin of the Siamese fighting fish, which has been utilized in breeding programs alongside ornamental betta fish to develop diverse species, color variations, shapes, and characteristics. This species is native to Southeast Asia, with several wild types of Siamese fighting fish documented [1]. Siamese fighting fish are popular as ornamental fish due to their vibrant colors and adaptability to small containers with poor-quality water [2]. However, the wild population of Siamese fighting fish is declining due to overexploitation, habitat disruption, and water pollution. Although Siamese fighting fish typically resist diseases, parasites, and bacteria, they can still be susceptible to various infections and diseases in natural habitats and aquaculture systems. [3-6]. Fish diseases caused by various pathogens, including parasites, bacteria, fungi, and viruses, pose a significant challenge in commercial fish farming [7]. The host-parasite relationship is influenced by environmental factors such as season, habitat, and fish behavior [7], and water quality parameters such as pH, temperature, salinity, and dissolved oxygen are affected by anthropogenic activities, further impacting fish health. The prevalence of parasites is strongly influenced by seasonal variations, and bacterial fish diseases also show seasonal patterns [8]. Parasites and bacteria are common culprits leading to illness, mortality, and reduced fish growth [7]. Parasitic infections affect fish in both wild and aquaculture environments across many world regions. Also, bacteria can be found in Siamese fighting fish and cause significant economic damage. The bacterial strains that are commonly encountered and cause harm include *Aeromonas hydrophila*, *A. caviae*, *Pseudomonas alcaligenes*, *Plesiomonas shigelloides*, *Vibrio fluvialis*, *V. cholera*, *Mycobacterium chelonae*, *M. cosmeticum*, *M. mucogenicum*, and *M. senegalense*. [5]. *Mycobacterium* spp. are among the most common bacterial pathogens. Mycobacterium infections, or Mycobacteriosis, are widespread and affect various internal organs, leading to decreased fish production and salability of ornamental fish. The infection can be passed on to the next generation and may even be zoonotic. Despite reported parasite, bacterial, and mycobacterial infections in Siamese fighting fish, no studies have compared wild and ornamental populations [9]. Understanding the diversity of parasites and bacteria is crucial for assessing the impact and causes of diseases, promoting responsible stewardship, and gaining insights into specific host infections. Moreover, this biological diversity data serves as a valuable biological index, reflecting changes in the physical ecosystem, including temperature, pH, salinity, dissolved oxygen, and water pollution, which fluctuate with the seasons. The diversity of parasites in fish accurately mirrors the diversity within the water source, providing valuable information about the effects and damages caused by pathogens and shedding light on the relationships between parasites and their hosts. Various parasitic infections are crucial biological indicators of environmental changes, offering insights into ecosystem health and water quality.

This study's objectives were to monitor the prevalence of parasites, observe their seasonal variation, assess bacterial infections in wild and ornamental Siamese fighting fish, and identify the risks of the disease and contamination to fish farming sites to serve as a guideline for finding treatment in the future. Awareness of native species conservation can influence the prosperity of society and pathogen infection factors. These findings were compared with wild populations collected from various stations. Increased awareness of native species conservation can positively impact social well-being and address pathogen infection factors.

2. Materials and Methods

2.1 Study area and collection of specimens

This study is directed towards examining the prevalence of diverse parasites and bacteria in *Betta splendens*, specifically ornamental Siamese fighting fish, obtained from commercial sources and natural freshwater environments in the central region of Thailand. The Research Ethics Committee and the Animal Care and Use Committee at King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand, approved this study on the bacterial isolation method from Siamese fighting fish (Certificate of Approval No. ACUC-KMITL-RES/2020/008). The sampling regime spanned twelve months, commencing in August 2020 and concluding in July 2021, encompassing the rainy, winter, and summer seasons. A total of 60 ornamental Siamese fighting fish, systematically selected at a rate of five specimens per month, were procured from the Chatuchak ornamental fish market in Bangkok throughout the study period. In the case of wild Siamese fighting fish, the specimens were acquired from three distinct sampling stations: station 1 (St01), situated in proximity to residential areas and canals (13°51′00.6″ N 100°28′05.4″ E); station 2 (St02), located near a community wastewater source (13°51′02.3″ N 100°28′05.4″ E); and station 3 (St03), positioned in a local orchard (13°51′05.7″ N 100°28′03.8″ E) (Figure 1). At each station, a maximum of five wild Siamese fighting fish samples were collected, albeit instances of acquiring fewer than five samples arose due to variable factors. pH and temperature data were recorded using a digital pH meter (AZ Instrumen, 8685) to assess the water quality at the sampling sites. Salinity levels were measured using a dedicated salinometer (ATC Refractometer Hydrometer), and dissolved oxygen content was determined using a dissolved oxygen test kit (v-color 9780, V-unique, U.S.A.). Subsequently, the collected Siamese fighting fish and water samples were meticulously transported in plastic box aquaria to maintain the vitality of the live specimens. Upon arrival, they were housed in a laboratory fish tank to facilitate subsequent examinations.

2.2 Identification of parasites

Measuring Siamese fighting fish involved recording their length (cm) and weight (g). Subsequently, pathological examinations were conducted to analyze the body surface mucus on all fins and the operculum. Thorough examinations were conducted on the gills, intestines, and internal organs to identify external parasites and endoparasite infestations originating from muscle tissues. A wet mount preparation technique was employed to observe parasites from the sampled Siamese fighting fish, and these parasites were then preserved in 70% alcohol. The examination and identification of parasites utilized a light microscope, with identification based on distinctive morphological features, as outlined in a comprehensive essential guide. The taxonomic classification was confined to genus series within trematode, nematode, acanthocephalan, protozoa, and monogeneans groups. Identification primarily relied on morphological characteristics of fresh specimens, as referenced in standard manuals and scientific papers. Notable sources included Buchmann and Bresciani [10] for protozoa and monogeneans, Bhattacharya [11] for acanthocephalan, Wagner [12] for Myxozoa, Khalil *et al.* [13] for cestode, Gibson *et al.* [14] for trematode, and Anderson *et al.* [15] for the nematode.

Figure 1. Sampling locations in central Thailand: St01 = Station 01, St02 = Station 02, St03 = Station 03.

2.3 Bacterial characterization

The Siamese fighting fish's kidneys, liver, and spleen were enriched in Trypticase soy broth (TSB) and incubated at 30 °C for 24 hours. Following incubation, a loopful was streaked on TSA plates and incubated at 30 °C for 24 hours. After incubation, a single colony representing various morphological types on each plate was selected and re-streaked onto a new TSA plate until a pure culture was obtained. Subsequently, morphological, physiological, and biochemical tests (including the API 20E system) were applied to characterize the isolated bacteria. To isolate *Mycobacterium* spp., the samples were enriched and incubated at 30 °C for 1-4 weeks. The enriched samples were then streaked onto Ogawa medium and incubated at 30 °C for 1-4 weeks. Acid-fast staining (Ziehl-Neelsen) was employed for characterization, and identification was achieved through biochemical tests using the API ZYM system.

Bacterial DNA was extracted for 16s rRNA gene sequencing. The 16s rRNA gene was amplified, and the PCR product was purified. Subsequently, DNA sequencing was performed using the standard Sanger method (U2Bio, Thailand). After obtaining the sequences, the forward and reverse reads were assembled using BioEdit version 7.2.5 software [16]. Species identification was conducted through Nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and the sequences were subsequently submitted to DDBJ (https://www.ddbj.nig.ac.jp/submission-e.html) under accession number LC853089.1 for *Aeromonas veronii* and LC853090.1 for *Mycobacterium marinum*. As both Gram-negative (*A. veronii*) and Gram-positive (*M. marinum*) bacteria were isolated, a phylogenetic tree was constructed using the Neighbor-Joining method [17] based on 16s rRNA gene sequences in MEGA12 software [18]. *Halobacterium salinarum* NBRC14715 (LC556329.1) was selected as the out-group for rooting the tree due to its distant phylogenetic relationship with both study organisms.

2.4 Data analysis

Descriptive analysis was employed to analyze parasites, their habitats, and their prevalence. The prevalence was determined by dividing the number of animals hosting a specific parasite by the total number of animals examined for a given parameter. The percentage (%) was also calculated to measure prevalence. Additionally, bacterial prevalence (%) was calculated by dividing the number of infected animals by the total number examined.

3. Results and Discussion

3.1 Siamese fighting fish measurement

During the period from August 2020 to July 2021, a total of 141 Siamese fighting fish were examined. This comprised 60 ornamental Siamese fighting fish obtained from the market and 81 wild Siamese fighting fish collected from three natural resource sites. The ornamental Siamese fighting fish exhibited an average body length of 5.40±0.86 cm and weights ranging from 0.96±0.42 g. In comparison, the wild Siamese fighting fish had an average body length of 3.74±0.43 cm and weights ranging from 0.64±0.23 g. The wild Siamese fighting fish were collected from three distinct stations. In St01, twenty fish samples were obtained, showcasing advantageous body lengths of 3.68±0.48 cm and weights ranging from 0.62±0.25 g. St02 comprised seventeen fish samples with body lengths averaging 3.53±0.39 cm and weights of 0.52±0.19 g. St03 included forty-four fish samples with body lengths of 4.02±0.42 cm and weights ranging from 0.79±0.25 g.

3.2 Parasite infection and water quality

Parasitological examination revealed that 15 out of 60 ornamental Siamese fighting fish were infected with parasites. The most prevalent was the monogenean *Dactylogyrus* sp. (25.00%), followed by the protozoan *Trichodina* sp. (1.67%). For the wild Siamese fighting fish, 28 out of 81 were parasitized (34.57%), presenting infection with six groups and seven genera of external and internal parasites. There were six groups and seven genera of the fauna of both external and internal parasites, including one genus of monogene (*Gyrodactylus* sp.), two genera of protozoa (*Trichodina* sp. and *Zoothamnium* sp.), one genus of Myxozoa (*Henneguya* sp. present plasmodium in gills and spores found on the body surface and specifically on the lesions present on the fish fins), one genus of nematode (*Proleptus* sp. present adult and cysts), one genus of trematode (*Posthodiplostomum* sp. present adult in muscle and metacercaria in the abdominal cavity), and a genus Pallisentis or spiny headed worm (*Pallisentis nagpyrensis* present cysts) were observed, as shown in Figure 2.

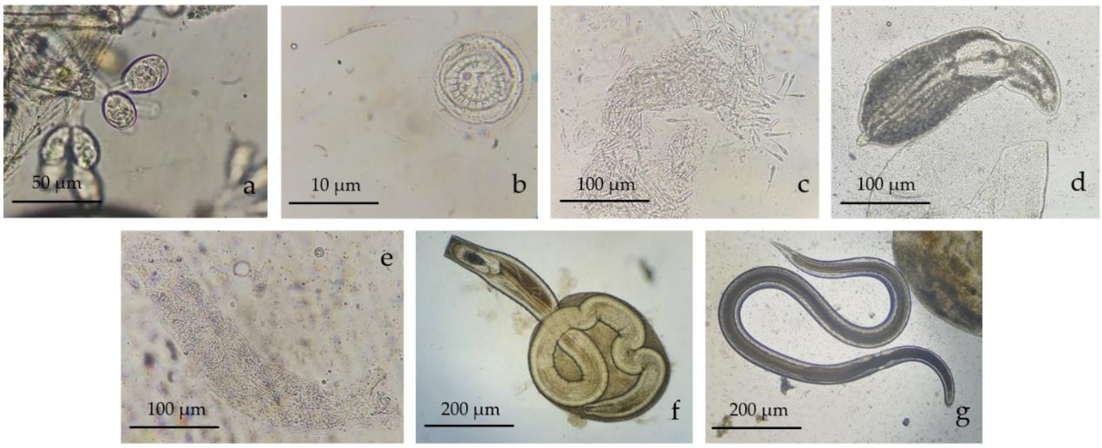

Figure 2. Parasite identification in wild *Betta splendens,* revealing the presence of *Zoothamnium* sp. (a), *Trichodina* sp. (b), *Henneguya* sp. (c), *Posthodiplostomum* sp. hatching from cyst (d), *Gyrodactylus* sp. (e), *Pallisentis* hatching from cyst (f), and *Contracaecum* sp. hatching from cyst (g).

In wild Siamese fighting fish, the highest prevalence (14.81%) was observed for *Trichodina* sp., followed by *Posthodiplostomum* sp. (11.11%), *Proleptus* sp. (9.88%), *Henneguya* sp. (8.64%), *Gyrodactylus* sp. (7.41%), *P. nagpyrensis* (1.23%), and *Zoothamnium* sp. (1.23%) (Table 1). The prevalence of parasites varied for each type among the 141 fish examined. Seasonal variations were noted, with both ornamental and wild Siamese fighting fish primarily infected with external parasites and higher prevalence occurring in the winter season. Ornamental fish showed similar parasite prevalence in summer (53.33%) and the lowest in the rainy season (6.67%). Wild fish exhibited a prevalence of 29.17% in the rainy season, followed by 52.94% in the winter season. *Trichodina* sp. had the highest prevalence in winter (29.41%), followed by the rainy (14.58%) season, and was absent in summer. *Henneguya* sp. (Figure 3.) infections were the second most common among external parasites and varied with the season. Among internal parasites, *Proleptus* sp. exhibited prevalence throughout the year, peaking in winter. *Posthodiplostomum* sp. appeared as both external and internal parasites throughout the year, with the highest cases in summer. Seasonal variations in infection for each parasite are detailed in Table 2. The examination of 81 wild Siamese fighting fish from three stations revealed 28 infected fish. The prevalence of infection varied among the stations, with St01 at 30.00% (6/20), St02 at 17.65% (3/17), and St03 at 43.18% (19/44). Seasonal prevalence of various parasites in wild Siamese fighting fish across different stations is provided in Table 3.

This research examines seasonal variations and environmental disparities in parasite infections among wild Siamese fighting fish. Clinical signs were identified by examining external, internal, and tissue abnormalities in wild Siamese fighting fish, comparing them to ornamental Siamese fighting fish. The study revealed a higher diversity and percentage prevalence of 34.57% in Siamese fighting fish in their natural habitat, in contrast to the low prevalence of 25.00% observed in ornamental Siamese fighting fish. Protozoa (*Trichodina*) and monogenean were the most common parasite genera recovered from ornamental and wild Siamese fighting fish, predominantly during winter. The results indicated seven genera of parasites, with *Trichodina* being the most prevalent, followed by *Posthodiplostomum*, *Proleptus*, *Henneguya*, *Gyrodactylus*, *Pallisentis*, and *Zoothamnium*, respectively. Research studies have consistently reported greater diversity and percentage prevalence of parasite infections that vary according to the season and location. *Trichodina* was predominantly found in winter but was absent in the summer season. Previous studies on parasites in ornamental *B. splendens*, such as Thilakaratne *et al.* [19], reported five species of parasites, including *Dactylogyrus* spp., *Gyrodactylus* spp., *Trichodina* spp., *I. multifiliis*, and *Piscionoodinium* spp. This report marks the first publication of the discovery of *Henneguya* infection occurring on the body surface (pelvic fin) and gills of wild Siamese fighting fish. In the case of infection from *P. nagpyrensis*, an internal parasite was found, and this occurrence was previously unreported. *Henneguya,* a common Myxospore, is highly host-specific, commonly infecting various organs or tissues [20]. Numerous infections have been documented on gill filaments, liver, kidney, spleen, heart, and muscle [21]. In the present study, *Henneguya* infection manifested as illness in specific wild Siamese fighting fish collected from St01 during the rainy season. While *Henneguya* was observed in all seasons at St03, its absence in St02 raises the possibility that water quality or pollution in this location might not support the viability of *Henneguya*. The prevalence of *Henneguya* infections was highest in winter (November to January), followed by the rainy season (May to October) and summer (February to April), respectively.

Several infections caused by Myxozoa have posed significant challenges in ornamental fish reared at breeding farms [22]. This parasitic infection may contribute to declining fish reproduction among wild Siamese fighting fish. In line with this report, a noticeable decrease in fish samples was observed at St01, which sometimes could not be collected during the last month of sampling. It can be inferred that the fish population is lower during the winter, summer, and early rainy seasons. Previous studies have provided limited insights into parasites infecting Siamese fighting fish. This study pioneers exploring parasite infections in wild Siamese fighting fish captured in natural freshwater, offering a detailed analysis of the relationship with seasonal variations. Comparative analysis of parasitic infection in wild Siamese fighting fish during seasonal changes revealed peak infections during transitions, such as the shift from winter to summer, the conclusion of winter in March, and the transition from the rainy season to winter in November. This highlights the significant influence of seasons on the parasite population.

Table 1. The overall prevalence of parasite infections in wild Siamese fighting fish and ornamental Siamese fighting fish

Fish examined	Parasites	No. of fish examined	No. of fish Infected	Prevalence (%)	Site of infection
Wild Siamese fighting fish	*Trichodina*	81	12	14.81	Body surface and gills
	Zoothamnium	81	1	1.23	Body surface
	Gyrodactylus	81	6	7.41	Gills
	Henneguya	81	7	8.64	Body surface and gills
	Posthodiplostomum	81	9	11.11	Gills, muscles, and Abdominal
	Pallisentis	81	1	1.23	Abdominal
	Proleptus	81	8	9.88	Abdominal
	Total	81	28	34.57	
Ornamental Siamese fighting fish	*Trichodina*	60	1	1.67	Gills
	Dactylogyrus sp.	60	15	25.00	Gills
	Total	60	15	25.00	

Winter exhibited the highest seasonal prevalence of investigated parasites, followed by the rainy and summer seasons. Each station's seasonal prevalence varied, with the highest parasite prevalence in winter (60.00%) observed in St03. In St01, the highest prevalence (36.84%) occurred only in the rainy season, while St02 recorded the highest prevalence (summer). Parasite prevalence varied according to seasons and stations, as shown in Table 4, indicating the influence of changing environmental parameters on the prevalence of different parasites in different seasons and stations. Examining water quality in March and November revealed parameter alterations, specifically salinity and dissolved oxygen (DO). Interestingly, this finding contrasts with Poulin's [2] observations, suggesting that certain parasites or hosts exhibit distinct infection peaks in the summer while others display no seasonal variations. The prevalence of parasitic infections may, in part, be attributed to the life cycle of each parasite, as indicated by previous reports. Neves *et al*. [23] investigated seasonal variation, noting that host attributes shape the parasite community structure of wild Oscar fish in the Brazilian Amazon. In Thailand, limited reports have associated parasite prevalence with seasonal variations in ornamental fish in natural water.

This research showcases samples of ornamental Siamese fighting fish exhibiting high parasite prevalence in February, April, and March, coinciding with dry weather conditions. The richness of parasite species could be attributed to environmental variability and differences along a latitudinal plot [24]. According to Adugna [25] research, a wide variety of parasites were identified, with the highest prevalence observed in lakes, surpassing that in farms and ponds. *Trichodina* spp. was detected predominantly in Nile tilapia (*Oreochromis niloticus*). In the case reported by Öztürk and Özer [26], *Aphanius danfordii*, the host of *Gyrodactylus* sp., showed higher prevalence in the spring season, followed by autumn, summer, and winter, respectively.

Seasonal environments affect water parameters, including pH, temperature, and conductivity, influencing parasitic infections in hosts. Temperature and low rainfall are imperative for parasite development [27]. The interaction and relationship between wild Siamese fighting fish size in each location and the prevalence percentages remain unclear. Notably, wild fish exhibited the largest average size in St03, followed by St01 and St02, but the percentages of prevalence and species diversity for parasites were highest in St03, followed by St02 and St01.

Table 2. The prevalence of infection of parasites in examined fish in different seasons

Season	**Fish examined**	**No. of fish examined**	**Parasite**	**No. of fish Infected**	**Prevalence (%)**
Rainy	Wild Siamese fighting fish	48	*Trichodina*	7	14.58
			Zoothamnium	1	2.08
			Gyrodactylus	4	8.33
			Henneguya	3	6.25
			Posthodiplostomum	4	8.33
			Proleptus	4	28.57
	Ornamental Siamese fighting fish	30	*Dactylogyrus*	2	6.67
Winter	Wild Siamese fighting fish	17	*Trichodina*	5	29.41
			Gyrodactylus	2	11.76
			Henneguya	3	17.65
			Posthodiplostomum	1	5.88
			Pallisentis	1	5.88
			Proleptus	2	11.76
	Ornamental Siamese fighting fish	15	*Dactylogyrus*	8	53.33
			Trichodina	1	6.67
Summer	Wild Siamese fighting fish	16	*Henneguya*	1	6.25
			Posthodiplostomum	2	12.50
			Proleptus	2	12.50
	Ornamental Siamese fighting fish	15	*Dactylogyrus*	8	53.33

Table 3. The percentage of parasite prevalence in wild Siamese fighting fish examined across different seasons and stations

Parasites	**Rainy**			**Winter**			**Summer**		
	St01 N=19	St02 N=5	St03 N=24	St01 N=1	St02 N=1	St03 N=15	St01 N=0	St02 N=11	St03 N=5
Trichodina	10.53	0	20.83	0	0	33.33	0	0	0
Zoothamnium	5.26	0	0	0	0	0	0	0	0
Gyrodactylus	5.26	0	15.79	0	0	13.33	0	0	0
Henneguya	5.26	0	8.33	0	0	20.00	0	0	20.00
Posthodiplostomum	15.79	0	4.17	0	0	6.67	0	9.09	40.00
Pallisentis	0	0	0	0	0	6.67	0	0	0
Proleptus	0	0	16.67	0	0	13.33	0	6.09	20.00
Total	36.84	0	33.33	0	0	60.00	0	27.27	40.00

Table 4. Parameters of water quality at each station

Season	Station	Temperature (°C)	Salinity (ppt)	pH	DO (mg/L)
Rainy	01	28.5	0.05	6.55	2.20
	02	27.6	0.06	6.62	1.70
	03	27.4	0.12	6.28	1.20
Winter	01	25.9	0.04	7.43	1.83
	02	25.9	0.07	7.59	1.33
	03	24.5	0.05	7.53	1.67
Summer	01	26.8	0.02	7.19	3.25
	02	26.5	0.04	7.58	1.75
	03	26.4	0.06	7.42	3.00

Figure 3. Normal wild *Betta splendens* (a). Wound in pelvic fin and pectoral fin of wild *B. splendens* (b). *Henneguya* sp. from fins of wild *B. splendens* (c).

3.3 Bacterial infection

The biochemical test, API 20E, and PCR detection resulted in pathogen identification isolated from ornamental Siamese fighting fish from the market. They revealed Gram-negative bacteria, *Aeromonas veronii*, Gram-positive bacteria, and the non-tuberculosis *Mycobacterium marinum*. Mycobacteriosis was investigated in the internal organs of ornamental Siamese fighting fish from markets, liver, spleen, and kidneys. At the same time, no infections were detected in the internal organs of wild Siamese fighting fish. The absence of *Mycobacterium* was confirmed by Ziehl-Neelsen acid-fast testing. This study encompassed both healthy and unhealthy ornamental Siamese fighting fish. Anomalous fish from the local farm exhibited clinical signs such as abdominal distention, big belly or dropsy (Figure 4a), and skin nodules (Figure 4b). Upon investigation of internal organ tissue, dark brown granulomas were identified in the ornamental Siamese fighting fish within a lighter capsule, observed through a wet mount biopsy (Figure 4c). One strain of Gram-negative bacteria (*A. veronii*) was identified in the case of wild Siamese fighting fish. Bacterial isolation targeted Siamese fighting fish's liver, spleen, and kidney. In wild Siamese fighting fish, *A. veronii* infections were detected fewer than in ornamental Siamese fighting fish. The identified bacteria included *A. veronii* and non-tuberculosis *M. marinum*. *M. marinum,* causing granulomatous disease or fish Mycobacteriosis, is known to occur in fish tanks and the culture of live feed for fish [28].

The findings and prevalence of bacterial infections in betta fish of this study revealed the presence of both *A. veronii* and *M. marinum* in ornamental Siamese fighting fish obtained from markets. In contrast, wild Siamese fighting fish showed only a limited presence of *A. veronii* (Table 5). This suggests higher bacterial diversity and infection prevalence in captive fish. Similar findings have been reported in cultured Striped knifejaw fish, where aquacultured populations showed greater gut bacterial diversity than wild fish, possibly due to environmental exposure and aquaculture practices [29]. Conversely, a study on common carp showed lower bacterial diversity in farmed fish than in wild ones [30]. These variations may reflect species differences and farming conditions. Our findings highlight the need for comprehensive comparative studies on microbial communities in cultured versus wild Siamese fighting fish populations to understand better the ecological and health implications of different rearing environments.

Table 5. Prevalence of bacterial infections in wild Siamese fighting fish and ornamental Siamese fighting fish

Bacteria	**Prevalence (%)**			
	wild Siamese fighting fish			ornamental Siamese fighting fish
	St01	St02	St03	
Aeromonas veronii	15	35.29	15.91	26.67
Mycobacterium marinum	0	0	0	10
Total	15	35.29	15.91	36.67

Dong *et al.* [5] researched the bacterial strains isolated from the skin nodule syndrome of ornamental Siamese fighting fish, identifying 23 different species. Four *Mycobacterium* strains were putatively identified as *M. chelonae*, *M. cosmeticum*, *M. mucogenicum* from the liver, and *M. senegalense* from the nodules. Mandrioli *et al*. [31] presented findings of granulomas in the spleen and other visceral organs of Cichlid fish infected by *M. chelonae* and *M. parascrofulaceum*. This study's results regarding *Mycobacterium* align with previous research, confirming the presence of *M. marinum* in the Siamese fighting fish, consistent with other reports identifying the most common *Mycobacterium* strains in betta fish as *M. marinum* and *M. fortuitum* [3, 32].

In the broader context of bacterial infections in ornamental Siamese fighting fish, studies have identified additional bacteria, including *A. hydrophila* [30], *Pseudomonas* spp., *Edwardsiella tarda*, and *Streptococcus* spp. [9] Walczak *et al.* [31] research, encompassing more than 182 bacterial isolates from ornamental fish, highlighted the prevalence of *A. veronii*, followed by *A. hydrophila*, *Shewanella putrefaciens*, and *Pseudomonas* spp., respectively. While this study reflects a diversity of bacteria similar to previous reports, it is noteworthy that the number of bacteria identified is relatively lower than reported in earlier studies. Previous findings have highlighted variations in bacterial strains contingent on fish species, habitat, and water quality. Specifically, investigations into aquaculture fish have underscored the impact of diverse culture methods on the array of bacterial strains present. Among these strains, *Mycobacterium*, a bacterial species with the potential for transmission through water sources and genetics, stands out. Peeler and Murry [35] detailed that pathogens and diseases originating in the aquaculture of farmed fish are often traced back to wild fish, creating a cycle where these issues can transmit from aquaculture fish to their wild counterparts and then circulate back into the aquaculture system.

In the context of the study area, it was discerned that the surrounding environment, characterized by sewage, pollution, and flooding, played a pivotal role in influencing the release of fish into natural water post-cultivation, thereby impacting the local economy. This environmental influence may contribute to the outcomes observed in the present study, where the prevalence and diversity of parasites and bacteria are shown to hinge on water source conditions and seasonal variations. The sampling protocols of the research, unavoidably influenced by factors like reproductive seasons, summer droughts, and rainy season floods, underscore the complexity of obtaining random fish samples. These uncontrollable elements further accentuate the intricate relationship between environmental dynamics and the study's observed prevalence and diversity of parasites and bacteria.

Figure 4. The clinical signs of ornamental Siamese fighting fish from the market include dropsy symptoms (a) and skin nodule symptoms (b). Additionally, granulomas found on internal organs due to infection in ornamental Siamese fighting fish can be seen in (c) as dark brown inside a lighter capsule visible on a wet mount biopsy.

Phylogenetic analysis revealed two major lineages. The first clade was divided into two subclades, supported by a strong bootstrap value of 97%. The first subclade included *A. veronii* WWKMITL-02 (LC853090.1) isolates separated into a distinct *A. veronii* strain subclade with a bootstrap value of 99%. The current *A. veronii* isolate was clustered among other *Aeromonas* spp. Isolates and distinctly separated from *Vibrio vulnificus*. The second clade included *M. marinum* WWKMITL-03 (LC853089.1) isolates grouped with *M. chelonae*, forming a strongly supported monophyletic group (99% bootstrap support). The phylogenetic relationships, based on 16s rRNA gene sequences of the bacterial isolates, which were submitted to DDBJ, are shown in Figure 5. Although Mycobacterium strains have been isolated from ornamental Siamese fighting fish in farms and markets [9], data regarding their occurrence in wild populations remains limited. Given their ability to persist in aquatic environments and transmit across generations, these infections may pose long-term ecological and economic risks, particularly to the ornamental fish trade. Proactive monitoring and biosecurity measures are essential to mitigate potential outbreaks and safeguard wild and cultured fish populations.

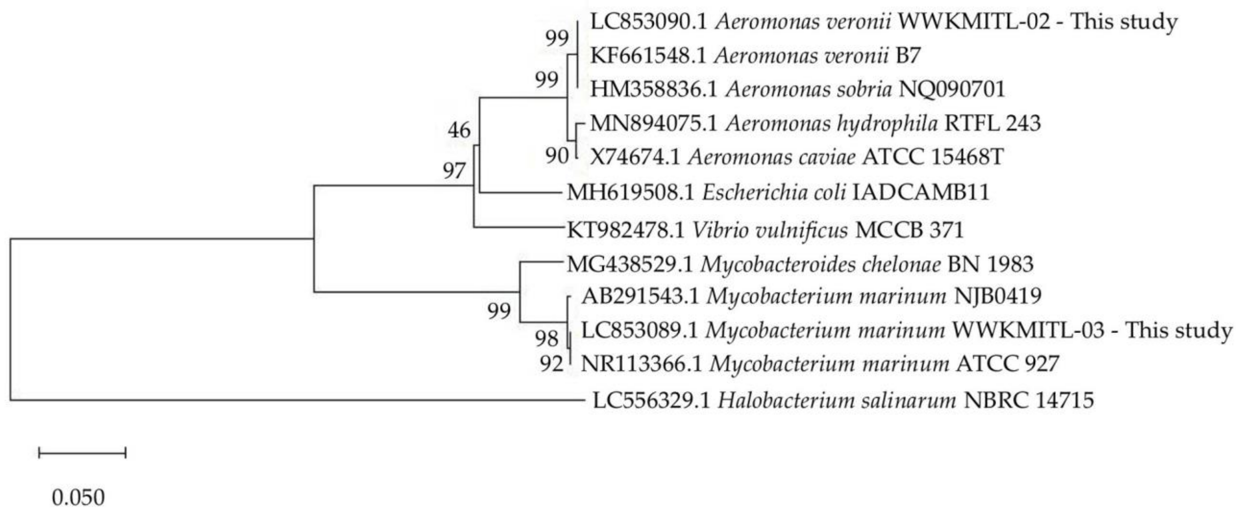

Figure 5. The phylogenetic relationship between two bacterial isolates, WWKMITL-02 (LC853090.1) and WWKMITL-03 (LC853089.1), was assessed using a tree generated via the neighbor-joining method based on 16s rRNA gene sequences. The Maximum Composite Likelihood method was applied to calculate nucleotide substitution distances. The tree was rooted with *Halobacterium salinarum* NBRC14715 (LC556329.1) as the outgroup. A scale bar indicating an evolutionary distance of 0.050 is provided to represent sequence divergence.

4. Conclusions

This study constitutes the inaugural investigation into the seasonal variations of parasitic infections among wild Siamese fighting fish within their natural water habitats, marking the first report of such observations. A distinctive facet of this research is the meticulous examination of *Henneguya* infection lesions on wild Siamese fighting fish fins, offering unprecedented insights into this hitherto unexplored dimension. The results illuminate a heightened prevalence and diversity of parasitic detection in wild Siamese fighting fish, eclipsing their ornamental counterparts. Significantly, the study discerns seasonal fluctuations in parasitic infection occurrences among wild Siamese fighting fish, underscoring the influence of water quality on both wild and aquaculture populations of Siamese fighting fish. After the rainy season, wild fish exhibit a zenith in parasitic prevalence and diversity, indicative of the conducive conditions for parasite growth during elevated temperatures.

In contrast, Siamese fighting fish from aquaculture systems and adhering to monthly or seasonal schedules exhibit negligible effects on parasite dynamics. The bacterial analysis further unravels a more excellent array of species isolated in ornamental Siamese fighting fish than in their wild counterparts, with notable identification of *M. marinum*, a genus linked to causing *Mycobacteriosis* infection. A comprehensive understanding of the complex interactions among seasonal variation, parasitic infections, and bacterial communities is crucial for elucidating the observed associations in wild fish populations. This pursuit aims to leverage such insights for preventing infections across diverse environments, agricultural landscapes, and potential human ramifications.

5. Acknowledgements

We gratefully acknowledge the financial support provided by King Mongkut's Institute of Technology Ladkrabang (KMITL) under Grant No. RA/TA-2563-D-002, which supported the Ph.D. study of PM, and Grant No. 2564-02-05-004 by the School of Science, KMITL.

Author Contributions: WW conceptualization, formal analysis, project administration, resources and writing-review and editing. SP bacterial analysis, interpretation of data analysis and writing-review. PM data curation, formal analysis, investigation, methodology, visualization and writing-original draft. All authors have read and agreed to the published version of the manuscript.

Funding: This work is financially supported by King Mongkut's Institute of Technology Ladkrabang (KMITL) under Grant No. RA/TA-2563-D-002 and Grant No. 2564-02-05-004 by the School of Science, KMITL.

Conflicts of Interest: We declare that we have no conflict of interest.

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